S12.18 A genetic system to study mitochondrial DNA mutations and their propagation in mice

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The number of mutations in mitochondrial DNA (mtDNA) thought to cause metabolic disorders in patients is growing. At present, we lack the technology to introduce these mutations into the mtDNA of model organisms for detailed study. In this study, random mtDNA mutations were generated using the transgenic mtDNA mutator mouse, which expresses an engineered mitochondrial polymerase defective in its proofreading function. Female lines were then established to transmit and segregate the resulting mutations. We observed the selective loss of nonsense mutations in the protein coding genes, consistent with strong purifying selection by the female germline. The selection was evident two generations after the founder mtDNA mutator mouse, eliminating these nonsense mutations before they were detectable by Sanger sequencing methods. Curiously, the tRNA and rRNA genes do not show the same rapid selection against mutations despite strong evolutionary sequence conservation. Despite this purifying selection, putative deleterious mutations were identified and propagated in the mouse lines. Also, mutations analogous to human pathogenic mtDNA mutations were identified. These results will aid in the generation of more accurate models of inheritance of disease-causing mtDNA mutations and allow for the generation of laboratory mouse models of mtDNA mutations known in human disease.

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S12.19 Adaptation of breast cancer cells to glucose deprivation: Increase in capacity and affinity of the oxphos system

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The aim of this study was to investigate the adaptation of cancer cells to low glucose conditions, as regard to their mitochondrial features. To mimick glucose deprivation we used the glutamine/galactose medium. We chose a model of human breast carcinoma (HTB-126) and control (HTB-125) cultured cells, as nearly 40% of breast malignancies exhibit hypoxic tumour regions with low oxygen concentrations (pO_2<2 mmHg) and poor glucose delivery. In these cells we measured the sensitivity of mitochondria towards a decrease in oxygen concentration by high resolution respirometry. This can be quantified by the p50 value, *i.e* the oxygen concentration at half-maximal respiration in intact cells. In cancer cells, glucose deprivation lead to a change in this apparent affinity for oxygen, as p50 values decreased from 0.62±0.13 μM (glucose) to 0.46±0.11 μM O_2 (galactose). There was also a 2 to 3-fold increase in routine respiration in glucose versus galactose medium, both in control and cancer cells, respectively. Mitochondrial network morphology also presented typical adaptations. These results suggest that in absence of glucose, as can occur in solid tumors, mitochondria are enhanced to produce the vital ATP.

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S12.20 Delayed assembly kinetics of respiratory chain complex I in cybrids harbouring primary LHON mutations

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Leber's hereditary optic neuropathy (LHON) constitutes the most frequent mitochondrial disorder. Over 90% of LHON cases are due to three point mutations in the mitochondrial *ND1*, *ND4* and *ND6* complex I genes. However, the functional effects of these mutations on complex I remain unsolved. By using blue native gel electrophoresis, we have studied complex I assembly in cybrids harbouring the three most common LHON mutations under different mitochondrial haplotypes. No decrease in complex I activity or in the steady-state levels of respiratory chain complexes was detected in the mutant cybrids. However, an accumulation of low molecular weight subcomplexes suggested an assembly or stability defect in the mutants. To check the assembly kinetics of respiratory chain complexes, cells were incubated for six days in the presence of a reversible inhibitor of mitochondrial translation, doxycicline. After this time the drug was released from the medium, which let assembly resume. Our results show delayed kinetics of complex I assembly and late recovery of complex I activity in all LHON cybrids compared to controls. Differences amongst cybrids carrying the same mutation under different haplogroups were observed. These results will provide essential information about the nature of complex I deficiencies and will enhance our understanding of LHON disease mechanisms.

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S12.21 Respiratory control and mitochondrial defects in the failing human heart

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Heart failure (HF) is a consequence of progressive deterioration of cardiac function. Little information is available on mitochondrial dysfunction in failing human hearts. We evaluated cardiac mitochondrial respiration in small biopsies from patients ranging from healthy donors to advanced stage of HF (dilated cardiomyopathy, during transplantation). Coupled OXPHOS capacity and uncoupled respiration (capacity of the electron transport system; ETS) were measured by high-resolution respirometry in permeabilized fibres (left and right ventricle, atrial appendage). ADP-stimulated flux through Complex I was only 0.4 of uncoupled respiration, indicating a strong limitation by the phosphorylation system. ETS capacity was higher with NADH-related substrates and succinate, supporting convergent electron input through Complexes I and II simultaneously into the Q-cycle. The additive effect of succinate was higher in patients with advanced stage of HF compared to donor hearts. HF mitochondria were tightly coupled, as evaluated by respiratory control in the absence of ADP. Fibres from atrial appendage showed lower respiratory fluxes with all substrate combinations compared to left and right ventricle. Respiratory control patterns (flux ratios for specific substrates relative to ETS capacity with convergent electron) were similar in the three tissues, but significantly different compared to the rodent heart. This study provides a basis for characterization of